PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P036460WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/000127	International filing date *(day/month/year)* 14.01.2004	Priority date *(day/month/year)* 14.01.2003

International Patent Classification (IPC) or national classification and IPC
C12Q1/68

Applicant
TISI, Laurence, C. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.11.2004	26.04.2005

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Hennard, C

Telephone No. +49 89 2399-7355

JC20 Rec'd PCT/PTO 06 JUL 2005

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000127

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-40	as originally filed
Claims, Numbers	
1-37	received on 29.03.2005 with letter of 29.03.2005
Drawings, Sheets	
1/12-12/12	as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000127

Box No. II Priority

1. ☒ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☒ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-33, 35
	No:	Claims	34, 36-37
Inventive step (IS)	Yes:	Claims	1-33
	No:	Claims	34-37
Industrial applicability (IA)	Yes:	Claims	1-37
	No:	Claims	None

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
   D1: WO 01/42496 A
   D2: ANALYTICAL BIOCHEMISTRY, vol. 288, no. 1, 1 January 2001, pages 28-38
   D3: WO 02/064830 A

The comments made by the applicant in his letter dated 15.11.2004 have been taken into consideration for issuing the present IPR.

2. **Novelty (Article 33(2) PCT):**
   D1 (page 14, line 17 - page 16, line 6; page 43, line 30 - page 44, line 33; figures 6-7; claims 1-11) and **D2** (page 30, right-hand column; page 32, left-hand column, lines 15-18) disclose a method for determining the amount of nucleic acid in a sample. The method involves mixing a target nucleic acid immobilised on streptavidin-coated beads, extension primers which hybridize to the target, and all the ingredients necessary for amplification of the target (polymerase...), APS, luciferin, ATP sulfurylase and luciferase. The measure of luminescence is made during the amplification using a luminometer. The amount of target nucleic acid is determined using quantitative standards. By modifying **claim 1** and specifying that the amplification (step ii) concerns the target nucleic acid and that more than one cycle of amplification are involved, novelty of independent **claim 1** is established.
   Considering the claimed kit, the documents **D1** and **D2** disclose processes which involve all the components of the kit of **claim 34**. Since from the wording of the claim the kit can be made of only one container (this is further justified by the dependent claim 35 which claims a kit made of two containers) this kit is anticipated by the processes of D1 and D2. Thus **claims 34 and 36** are not novel.
   Moreover, the device for performing the method can be represented by a luminometer disclosed in **D1** and **D2** which both disclose a process involving all the elements of the kit of claim 34, therefore **claim 37** is not new.
   In the light of these documents, **claims 34, 36 and 37** are not new and do not fulfil the requirements of **Article 33(2) PCT**.

3. **Inventive merit (Article 33(3) PCT):**
   3.1 **D1** (same passages as above), which is the closest prior art, concerns a

method for assessing the amount of nucleic acid in a sample involving the measurement of pyrophosphate produced during amplification by means of bioluminescence. The method of **claim 1** of the present application distinguishes itself from the prior art by the amplification which involves more than one amplification cycle.

By performing the process involving more than one amplification cycle, the process of the present **claim 1**, according to the applicant's statement made in his letter dated 15.11.2004, overcomes the prejudice that the elements of the bioluminescence detection are unstable over a longer time period (more than one minute). Therefore, the problem to be solved can be seen in the provision of a method for determining the amount of template nucleic acid present in a sample involving a multiple cycle amplification without loss of bioluminescence activity.

The skilled person would not, knowing that the bioluminescent elements are unstable over time, suggest a method in which multiple amplification cycles are performed. Thus an inventive merit can be recognised for independent **claim 1**.

3.2 Dependent **claim 35**, relating to a kit made of two containers, one containing the ingredients for amplification and the other the ingredients for the bioluminescence is considered not inventive considering the disclosure of **D1** (page 44 and claims 22-23) in combination with **D3** (claim 17).

3.5 In order to summarise the above objections, **claims 1-33** are considered to involve an inventive merit and to fulfil the requirements of **Article 33(3) PCT**, whereas **claim 35** is not inventive.

4. **Industrial applicability (Article 33(4) PCT):**
Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-37** of the present application are considered to fulfil the requirements of **Article 33(4) PCT**.

5. Contrary to the requirements of **Rule 5.1(a)(ii) PCT**, the relevant background art disclosed in **D1 and D2** is not mentioned in the description, nor are these documents identified therein.

CLAIMS:

1. A method for determining the amount of template nucleic acid present in a sample comprising the steps of:

i) bringing into association with the sample all the components necessary for nucleic acid amplification, and all the components necessary for a bioluminescence assay for nucleic acid amplification including:

a) a nucleic acid polymerase,

b) the substrates for the nucleic acid polymerase,

c) at least two primers,

d) a thermostable luciferase,

e) luciferin,

f) optionally ATP sulphurylase, and

g) optionally adenosine 5' phosphosulphate,

and subsequently:

ii) performing a nucleic acid amplification reaction of the target nucleic acid involving more than one cycle of amplification;

iii) monitoring the intensity of light output from the bioluminescence reaction, and

iv) determining the amount of template nucleic acid present in the sample.

2. A method according to claim 1, wherein at least steps ii) and iii) are carried out in a sealed vessel.

3. A method according to claim 1 or claim 2, wherein in step iii) the intensity of light output is monitored during the nucleic acid amplification reaction.

4. A method according to any one of claims 1 to 3, wherein step iii) further includes producing a data set of intensity of light output as a function of time.

5. A method according to any one of claims 4 to 6, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of change of intensity of light output changes significantly.

6. A method according to any one of claims 1 to 4 for determining the amount of template nucleic acid present in the sample at the beginning of the nucleic acid amplification reaction of step ii).

7. A method according to any one of claims 1 to 4 for determining the amount of template nucleic acid present in the sample as a result of the nucleic acid amplification reaction of step ii).

8. A method according to any one of claims 5 to 7, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output begins to increase.

9. A method according to any one of claims 5 to 7, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output is at a maximum.

10. A method according to any one of claims 5 to 7, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output increases.

11. A method according to any one of claims 5 to 7, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output decreases.

12. A method according to any one of claims 5 to 7, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output reaches or crosses a predetermined level.

13. A method according to any one of claims 8 to 12, wherein the thermostable luciferase that is brought into association with the sample in step i) is a reversibly-inhibited luciferase.

14. A method according to any one of the preceding claims, wherein step iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which the sample comprises a known amount of template nucleic acid.

15. A method according to any one of claims 1 to 14 for determining whether the template nucleic acid is present in the sample.

16. A method according to claim 14, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data set whether the intensity of light output reaches or crosses a predetermined level.

17. A method according to claim 15, wherein an increase in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

18. A method according to claim 15, wherein a decrease in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

19. A method according to any one of claims 16 to 18, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data set whether the intensity of light output reaches or crosses the predetermined level within a predetermined length of time following the start of the amplification reaction of step ii).

20. A method according to any one of the preceding claims, wherein step iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which no amplification has taken place.

21. A method according to claim 20, wherein a decrease in the intensity of light output relative to a control reaction in which no amplification has taken place indicates the presence of template nucleic acid in the sample.

22. A method according to any one of claims 1 to 21, wherein the nucleic acid amplification reaction of step ii) is a low temperature thermocycling amplification method in which the cycling temperature range does not exceed 75°C.

23. A method according to any one of claims 1 to 21, wherein the nucleic acid amplification reaction of step ii) is carried out isothermally.

24. A method according to claim 23, wherein the nucleic acid amplification reaction of step ii) is carried out within a temperature range that does not exceed 75°C.

25. A method according to claim 23 or claim 24, wherein the nucleic acid amplification reaction of step ii) is carried out at a constant temperature at which the components of the amplification reaction and the bioluminescence assay are stable.

26. A method according to claim 23 or claim 24, wherein the nucleic acid amplification reaction of step ii) is carried out at more than one temperature within the temperature range in which the components of the amplification reaction and the bioluminescence assay are stable.

27. A method according to claim 26, wherein the nucleic acid amplification reaction of step ii) is started at a higher temperature and subsequently dropped to a lower temperature.

28. A method according to any preceding claim for use in medical diagnostics.

29. A method according to any preceding claim for use in determining whether a pathogen is present in a sample.

30. A method according to any preceding claim for determining whether a particular nucleic acid sequence is present in an organism's genetic code.

31. A method according to claim 30 for determining whether the nucleic acid to which the template nucleic acid originates has been genetically modified.

32. A method according to any one of claims 1 to 27 for determining whether an organism is present in a sample.

33. A method according to any one of claims 1 to 27 for use in immuno-nucleic acid amplification technology.

34. A kit for use in a method according to any one of the preceding claims, wherein the kit comprises a nucleic acid polymerase, the substrates for the nucleic acid polymerase, at least two primers, a thermostable luciferase, luciferin and optionally ATP sulphurylase and adenosine 5' phosphosulphate.

35. A kit for use in a method according to any one of claims 1 to 33, wherein the kit comprises containers respectively containing:

a) a buffered mixture of nucleic acid polymerase, a source of Mg and dNTPs; and

b) a luciferase, luciferin and ATP sulphurylase.

36. A kit according to claim 34 or claim 35, wherein at least one of the components of the kit is in a form which is suitable for storage in the kit.

37. A device for performing a method according to any one of claims 1 to 32, wherein said device incorporates the components that are present in a kit according to any one of claims 34 to 36.